RESEARCH ARTICLE

Prevalence of Subclinical Coccidiosis in Broilers

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Abstract

The present study was carried out to assess the prevalence of subclinical coccidiosis in broilers affected with different diseases/ pathological conditions received for post-mortem examination. The study was carried out in 50 broiler flocks of 2-7 weeks age. The study comprised of molecular detection and oocyst count (OPG) of subclinical coccidiosis. Samples were collected from three birds in each flock and observed by direct microscopic method. Out of 50 flocks investigated, *Eimeria* oocysts were detected in 17 (34%) broiler flocks by direct smear examination. On molecular detection of *Eimeria* species in 50 flocks, 19 (38%) were found positive in which *E. tenella* and E. *acervulina* were detected. Among the 50 flocks tested *Eimeria* species encountered were *E. tenella* and *E. acervulina* in 30% and 8%, respectively.

Key words: Coccidiosis, Eimeria, Molecular detection, Prevalence, Subclinical.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.1.32

Introduction

Indian poultry industry has emerged as the most dynamic and fast expanding segment of agro-animal based industry. Various infectious diseases, parasitic infections have an important role causing hidden economic losses in the production of poultry meat and egg with deleterious and debilitating effects on infected birds, especially young birds causing decreased feed conversion, retarded growth, interfering development and making them susceptible to secondary infections (Adang *et al*., 2014). Infected birds tend to huddle together, have ruffled feathers and show signs of depression. The birds consume less feed and water, and the droppings are watery to whitish or bloody. This results in dehydration and poor weight gain as well as mortalities.

The lesions of coccidiosis depend on the degree of inflammation and damage to the intestinal tract. These include thickness of the intestinal wall, mucoid to blood-tinged exudates, petechial haemorrhages, necrosis and mucous profuse bleeding in the caeca, primarily causing severe enteritis and typhilitis with haemorrhages, tissue damage and severe metabolic disturbances (Rajkhowa *et al*., 2012). When oocysts are ingested by a chicken, the sporozoites are liberated by mechanical abrasion of the oocyst wall in the chicken gizzard followed by enzymatic digestion of the sporocyst wall in the lumen of the upper intestine. Sporozoites migrate to their preferred sites of development where they invade villus enterocytes. *E. tenella*, develop within enterocytes of the crypts. The accurate identification of *Eimeria* species has important implications for diagnosis and disease control, but also to the epidemiology and biology studies, creation of new vaccines and selection of anticoccidial drugs. The present study was carried out to study the prevalence of subclinical coccidiosis, oocyst load and molecular detection of *Eimeria* species in broiler chicken.

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How to cite this article: Prajapati, R. K., Ghodasara, D. J., Bhanderi, B. B., & Gehani, M. T., (2026). Prevalence of Subclinical Coccidiosis in Broilers. Ind J Vet Sci & Biotech, 22(1), 165-169.

Source of support: Nil

Conflict of interest: None

Submitted 08/12/2025 **Accepted** 15/12/2025 **Published** 10/01/2026

Materials and Methods

During January 2019 to June 2019, a total of 50 commercial broiler poultry farms were sampled from Anand district, Gujarat (India). The study was carried out in commercial broiler birds affected with different diseases/pathological conditions received for postmortem examination at the Department of Pathology, Veterinary College, AAU, Anand. The samples were collected in 10% neutral buffered formalin from 2-7 weeks of dead broiler birds affected with different diseases/pathological conditions for histopathology.

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Counting of Oocyst by McMaster Technique

Two grams of fecal sample weighed into a cup on scale was dispensed in 28 mL flotation solution into the beaker, mixed and was let soak for approximately 5 min. It was strained with a tea strainer into the second beaker pressing fluid through with the tongue depressor. Immediately, both chambers of the McMaster slide (Eggzamin, USA) were filled using a transfer pipette. After 5 min the McMaster slide was placed onto the microscope stage, counted all eggs inside of the grid areas using the 10X objective in both chambers. Eggs in both chambers were counted. Total egg count: (chamber 1 + chamber 2) x 50 = eggs per gram (EPG). This multiplication factor of 50 is specific to the ratio of feces (2 grams) to flotation solution (28 mL) described in this procedure (Zajac and Conboy, 2012).

Pathological Studies

Detailed post-mortem examination of carcasses was carried out and gross lesions were recorded of the flocks included in the study. Histopathological examinations of intestine and caeca preserved in 10% neutral buffered formalin were processed by paraffin embedding method. Sections were cut at 5-6 micron thickness with the help of microtome and stained with Ehrlich's haematoxylin and eosin (H&E) method for examinations as described by Luna (1968).

Molecular Characterization of *Eimeria* Spp.

Frozen faecal samples were thawed and processed for extraction of the DNA for further identification of *Eimeria* spp. by PCR. DNA was extracted from all the different pooled samples by QIAamp®DNA Stool Mini Kit as per protocol (Qiagen, Germany). Quantification and quality assessment, absorbance was read in Nanodrop Spectrophotometer at 260 nm. The DNA from fecal samples was quantified and the ratio of purity was evaluated following the method of Sambrook and Russel (2001). The pairs of primers (Eurofins Genomics India Pvt Ltd, India) used for PCR amplification were as per the details given in Table 1 (Bhaskaran *et al*., 2010). The composition of PCR reaction and steps and cycling conditions for PCR for detection of *Eimeria* spp. is mentioned in the Table 2 and 3, respectively.

Table 1: Primer sequence for *Eimeria* spp.

Primer Name	Sequence (5′– 3′)	Product size
E. acervulina	F: GGCTTGGATGATGTTTGCTG	321 bp
	R: CGAACGCAATAACACACGCT	
E. maxima	F: GCGGTTTCATCATCCATCATCG	145 bp
	R: CGTTGTGAGAAG/AACTGA/GAAGGG	
E. necatrix	F: TACATCCCAATCTTTGAATCG	383 bp
	R: GGCATACTAGCTTCGAGCAAC	
E. tenella	F: AATTTAGTCCATCGCAACCCT	278 bp
	R: CGAGCGCTCTGCATACGACA	

Table 2: Quantity and concentration of various components used in PCR

Components	Quantity	Final Conc.
DNase-RNase free milliQ water	7.50 µL	–
2X PCR master mix (Emrald)	12.50 µL	1 X
Forward Primer (10 pmol/µl)	1.00 µL	10 pmol
Reverse Primer (10 pmol/ µl)	1.00 µL	10 pmol
DNA template	3.00 µL	–
Total	25.00 µL	–

Table 3: Steps and cycling conditions for PCR for detection of *Eimeria* spp

	Initial De-naturation	Denatura-tion	Anneal-ing	Exten-sion	Final Exten-sion
Temperature	94 °C	94°C	55°C	72°C	72°C
Time	3 min	30 sec.	30 sec.	1 min. 30 sec.	7 min.
			30 cycles		

To confirm the targeted PCR amplification, 5 µL of the PCR products from each tube was electrophoresed along with DNA molecular weight marker (HiMedia Pvt. Ltd, India) on 2.0% agarose (Low EEO, SeaKem) gels containing 0.5 µg/mL ethidium bromide (Sigma-Aldrich, USA) at 80 V and 400 mA for 45 min in 0.5X Tris Borate EDTA (TBE) buffer. The amplified product was visualized as single compact band of expected size under UV light and was documented by gel documentation system (Genetix Biotech Pvt. Ltd, India).

Results and Discussion

Oocysts Count

All eggs inside the grid areas were counted using the 10X objective in both chambers of McMaster slide (Fig. 1). Oocysts were observed in 17 (38%) flocks out of 50 flocks by direct smear and OPG (oocysts per gram of feces) was counted for birds aged 18-46 days. Lowest oocyst count was found in flock no. 12 (1600 OPG), while highest oocyst count was found in flock no. 45 (63,950 OPG). Mean OPG in positive broiler flocks was 22,229.41. The number of oocysts multiplied by 50 represented the number per gram of the sample collected. Haug *et al*. (2008) indicated that risk flock may be used as an indicator of likely production loss in a broiler population. Karaer *et al*. (2012) counted mean OPG in the samples as 36,498.7(50 to 952,000). Menon *et al*. (2011) recorded OPG of fecal sample in all the positive samples, to be in range of 50 to 75,000 and out of 47 positive samples 31.91%, 31.91%, 14.8% and 21.2% showed mild, moderate, severe and very severe infection, respectively, while in present study out of 17 samples 00%, 23.52%, 23.52%, 52.94% samples showed mild, moderate, severe and very severe infection, respectively.

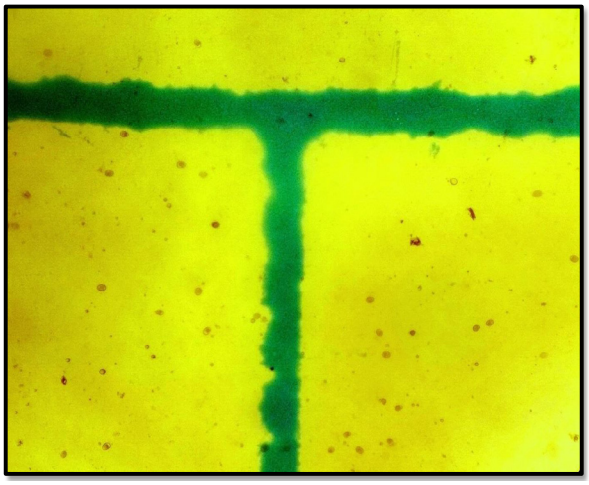

Fig. 1: Oocysts counting by using McMaster slide under the 10X objective.

Pathological Findings

Birds from all fifty flocks were examined for gross lesions in the intestine. Most of the birds revealed normal intestinal mucosa and normal intestinal content. Few birds revealed slight thickened mucosa with presence of light-yellow mucus exudate. The caecal content was light brown in colour and semi-liquid in consistency in most of the birds. Few birds showed slight dark brown colour and little hard caecal content. A total of 17 (34%) flocks were positive for subclinical coccidiosis by direct microscopic examination of intestinal and caecal content. Microscopic lesions observed in 10 flocks which were also found positive by direct smear examination for subclinical coccidiosis. Lesions in caecum which were positive for *E. tenella* showed haemorrhages in mucosa and submucosa of epithelial glands, cellular infiltration and necrosis of glands in few birds (Fig. 2). Lesions in cecum of broiler bird with subclinical *E. tenella* infection showed colony of oocysts, hemorrhagic areas in submucosa and destructed mucosal gland (Fig. 3). The duodenal mucosa showed damage to the epithelium. Adamu *et al.* (2013), Debbou-Iouknane *et al.* (2018), Shahraki *et al.* (2018) carried out histopathological examination of intestine and revealed various changes in the sections of both the caeca and the small intestine affected with coccidiosis, such as loss of epithelial tissue, presence of oocysts in the epithelial cells and infiltration of various cells in the intestine. Similar lesions were also found in the present study.

Molecular Characterization of *Eimeria* Spp.

The results of molecular detection of *Eimeria* oocyst revealed that out of 50 samples, 19 (38%) were found positive for presence of oocysts. In present study, genomic DNA of *Eimeria* was extracted from intestinal and caecal content of 50 broiler flocks by QIAamp®DNA Stool MiniKit (Qiagen, Germany). The amplified PCR products from different samples run in 2 % agarose gel, indicated the corresponding amplified band in different isolates (Fig. 4, 5). During the study it was found that two species, *viz., E. tenella* and *E. acervulina* were successfully detected from 50 samples. The frequency of species was variable in different flocks, where *E. tenella* was detected in 15 (30%) flocks, while occurrence of *E. acervulina* was found in 4 (8%) flocks.

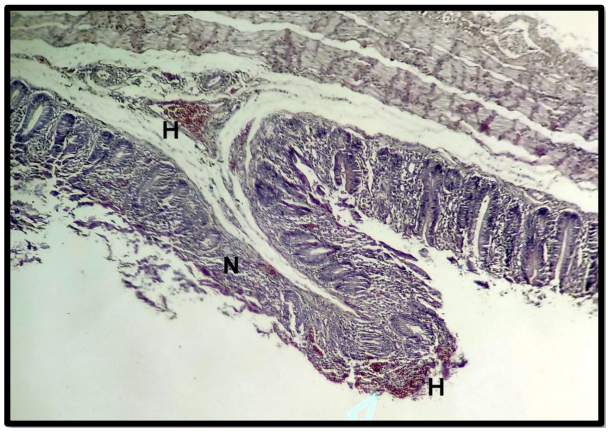

Fig. 2: Section of the cecum of broiler bird with subclinical *E. tenella* infection showing haemorrhage in mucosa and submucosa (H), cellular infiltration and necrosis of glands (N) (H & E stain, 240X).

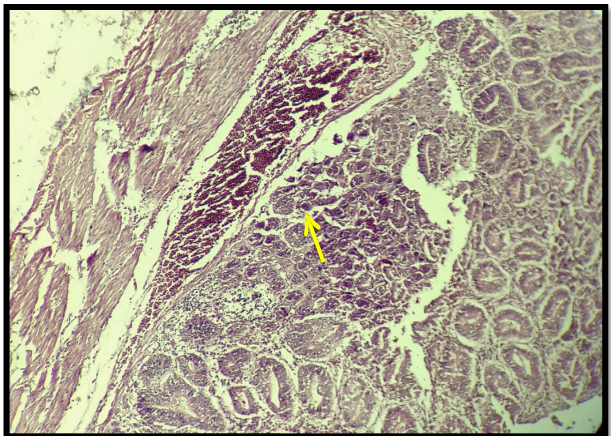

Fig. 3: Section of the cecum of broiler bird with subclinical *E. tenella* infection showing colony of oocysts and hemorrhagic areas in submucosa, (H & E stain, 240X).

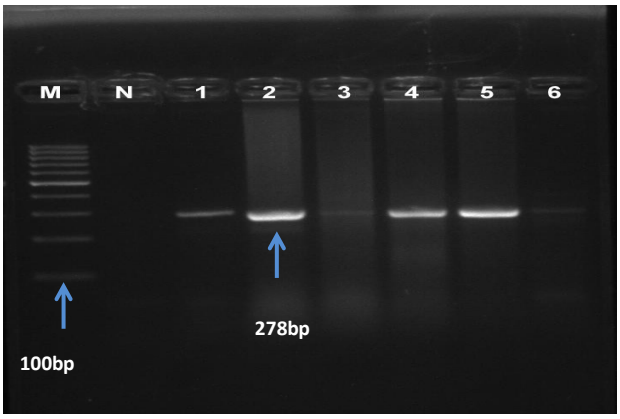

Fig. 4: Agarose gel image showing amplified PCR product of 278 bp of *E. tenella*. M: DNA ladder-100 bp, 1-6: Representative positive samples, N: Negative control

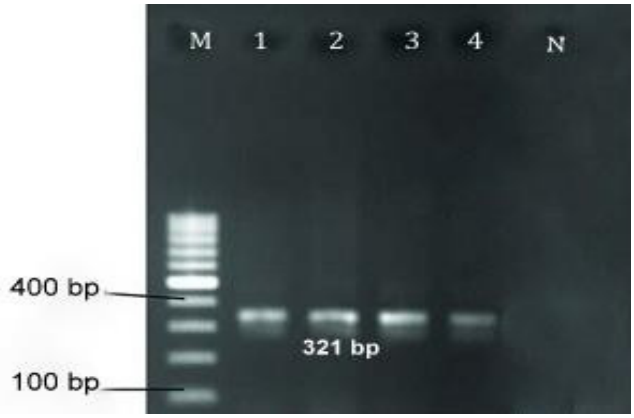

Fig. 5: Agarose gel image showing amplified PCR product of 321 bp of *E. acervulina*. M: DNA ladder- 100 bp 1-4: Positive samples, N: Negative control

Overall, the most prevalent species was *E. tenella*, which was detected in 30% flocks. The second most prevalent species was *E. acervulina* which was present in 8% flocks. Mixed infection with two or more *Eimeria* species was not found in any of the flocks. Out of the four *Eimeria* species, DNA representing two species (*E. acervulina* and *E. tenella*) were detected in the fecal samples from broiler farms. The two remaining species (*E. necatrix* and *E. maxima*) were not detected in feces from any of the sampled flocks. Traditional methods have major limitations in the specific diagnosis of coccidiosis; there have been significant advances in the development of molecular diagnostic tools. Species-specific PCR was successfully used to determine four of the seven recognized *Eimeria* species. Two out of four the identified species (*E. acervulina* and *E. tenella*) are considered to be pathogenic or highly pathogenic in poultry.

It was important to note that moderate to low amounts of parasite DNA were identified in the majority of fecal samples collected from the broiler flocks and no gross lesions of *Eimeria* infection were observed in the birds at the time of sampling. Subclinical infection can lead to severe economic losses from reduced weight gain and increased feed conversion ratios in affected birds. Intensive chicken farming across the world depends on specific prophylaxis to control coccidiosis within-feed anticoccidial drugs and in some market's live vaccines. Drug-resistant *Eimeria* strains are responsible for subclinical coccidiosis and resultantly for reduced broiler performance (Shirzad *et al.,* 2011). Poor evaluation of parasitological method to identify the *Eimeria* species has been reported previously by Haug *et al*. (2008). The present study also showed that there was good agreement between PCR and traditional detection of *Eimeria* species. Razmi and Kalideri (2000) reported 38% farm level prevalence of subclinical coccidiosis with peak oocyst score in the litter at more than six weeks of age. Chalchisa and Deressa (2016) reported 39.58% flocks were positive for coccidian oocysts. Most farms had *E. acervulina, E. maxima* and *E. tenella*. Many workers have reported lower prevalence of subclinical coccidiosis than present finding (Ahmed *et al*., 2009; Jatau *et al.,* 2012, Oljira *et al.,* 2012, Ali *et al.,* 2014; Ahad *et al.,* 2015; Tesfaye *et al.,* 2015; Lawal *et al.,* 2016; Lan *et al*., 2017; Hamid *et al*., 2018).

Conclusion

The study demonstrated that coccidiosis remains a significant health concern in broiler flocks, with *Eimeria* infections detected both clinically and sub-clinically across multiple farms. Direct smear examination and PCR confirmed the presence of *E. tenella* and *E. acervulina,* with *E. tenella* being the predominant species. High oocyst counts and characteristic gross and histopathological lesions further supported active infection in affected flocks. Overall, the findings highlight the need for effective monitoring, timely diagnosis, and robust preventive strategies to reduce economic losses and improve broiler health and production efficiency.

Acknowledgement

The authors acknowledge the invaluable support and guidance provided by the Department of Veterinary Pathology, Veterinary College, Anand, during the course of this research work. Sincere gratitude goes to all the faculty members whose encouragement, facilities, and thoughtful suggestions greatly contributed to the successful completion of this study.

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